

-2-

Serial No. 09/998,631

**AMENDMENTS TO THE CLAIMS**

This listing of claims will replace all prior versions and listings of claims in the application:

**Listing of Claims:**

1. (Original) A method of producing a recombinant peptide, a recombinant protein or a product from metabolic engineering using a genetically modified first methylotrophic bacterium under the control of a regulated promoter from a second methylotrophic microorganism of the same or different species; comprising the steps of:

- (a) introducing into said first methylotrophic bacterium an expression vector comprising a polynucleotide sequence, encoding for a peptide or a protein or allowing production of a product from metabolic engineering, under the control of a regulated promoter;
- (b) growing said genetically modified first methylotrophic bacterium in a minimal salts medium lacking organic sugars and containing methanol for a time sufficient to allow production of said peptide or protein or said product from metabolic engineering; and
- (c) regulating expression of said polynucleotide sequence by said promoter.

2. (Original) The method of claim 1, wherein said regulated promoter is a metal regulated promoter.

-3-

Serial No. 09/998,631

3. (Original) The method of claim 2, wherein step c) is effected with a metal ion.

4. (Original) The method of claim 1, wherein said first and/or second methylotrophic bacterium is a facultative methylotroph or an obligate methylotroph.

5. (Original) The method of claim 1, wherein said first methylotrophic bacterium is of the species *Methylobacterium*.

6. (Original) The method of claim 1, wherein said first methylotrophic microorganism is *Methylobacterium extorquens* ATCC 55366.

7. (Original) The method of claim 1, wherein said polynucleotide sequence is a gene encoding for green fluorescent protein.

8. (Original) The method of claim 1, wherein said polynucleotide sequence is a gene encoding for an enzyme.

9. (Original) The method of claim 8, wherein said enzyme reacts with a component within or from said culture medium to produce a biomaterial or a product from metabolic engineering.

10. (Original) The method of claim 1, wherein said peptide or protein or said product from metabolic engineering reacts with a component within or from said culture medium to produce a biomaterial.

-4-

Serial No. 09/998,631

11. (Original) The method of claim 1, wherein said polynucleotide sequence is inserted into a vector suitable for introduction into a methylotrophic microorganism.

12. (Original) The method of claim 11, wherein said vector is capable of reproduction within said bacterium and said vector is stably maintained within said bacterium during growth and replication of said bacterium, in presence of selective pressure.

13. (Original) The method of claim 12, wherein said selective pressure is an antibiotic.

14. (Original) The method of claim 11, wherein said vector1 allows for the expression of said polynucleotide sequence within said methylotrophic bacterium.

15. (Original) The method of claim 3, wherein said metal ion is  $\text{Cu}^{2+}$ .

16. (Original) The method of claim 1, wherein said promoter is the promoter present in the soluble methane monooxygenase (sMMO) operon of *Methylosinus trichosporium* OB3b.

17. (Original) The method of claim 3, wherein said vector is *pmmoX*-GFP-pRK310.

18. (Original) The method of claim 3, wherein said vector is *pmmoX*-GFP-pVK101.

-5-

Serial No. 09/998,631

19. (Original) The method of claim 1, further comprising the step of:  
(d) controlling the expression of said polynucleotide sequence with a promoter from a gene from an organism other than a methylotrophic bacterium.
20. (Original) The method of claim 19, wherein said vector is *pLac-GFP-pJB3KmD*.
21. (Original) The method of claim 19, wherein said vector is *pLac-GFP-pRK310*.
22. (Original) The method of claim 1, wherein the use can be for high-throughput production of a peptide, protein or product from metabolic engineering.
23. (Original) The method of claim 1, wherein the use can be for proteomics-based peptide or protein expression.
24. (Original) The method of claim 12, wherein said growth and replication of said bacterium is performed within a flask or fermenter.
25. (Original) The method of claim 1, wherein said protein is a polypeptide > 10 amino acid residues in length.
26. (Original) The method of claim 1, wherein said peptide is  $\leq 10$  amino acid residues in length.

-6-

Serial No. 09/998,631

27. (Amended) An expression vector for producing a recombinant peptide, a recombinant protein or a product from metabolic engineering in a methylotrophic bacterium, wherein said expression vector is as defined in claim 1 ~~comprises a polynucleotide sequence encoding for a peptide or a protein or allowing production of a product from metabolic engineering, under the control of a metal regulated promoter.~~